

The Use of Antibiotics and Gamma Irradiation in the Aging of Steaks at High Temperatures^{a, b, c}

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IN THE MEAT INDUSTRY, the aging of beef carcasses and cuts to improve tenderness is frequently carried out by holding the meat for two weeks at 35° to 40° F. This procedure requires large inventories and results in moisture and trimming losses. For these reasons beef usually moves directly to the retail outlet without aging. A rapid, economical method of aging would permit more beef, particularly the lower grades, to be tenderized in this manner.

Assuming that the tenderization which occurs during aging is an enzymatic process, it follows that the aging time can be reduced if the temperature is raised. Weiser *et al* (9) aged beef rounds and carcasses at room temperature (75–85° F) after infusion with an antibiotic solution and found them to be more tender than controls which had been chilled after slaughter. In other experiments aging beef for 2–3 days at temperatures ranging from 57° to 86° F, produced tenderness changes similar to those found in beef aged 12–14 days at 35° to 40° F (6, 7). In this work ultraviolet irradiation was used to control surface microbial growth.

In the present study tetracycline antibiotics alone and in combination with gamma radiation were used as preservatives during aging at elevated temperatures. Niven and Chesbro (4) have reported that high energy irradiation and the tetracycline antibiotics complement each other in their preservative effect on meat. The adverse effects of irradiation on meat flavor are generally proportional to the dosage employed but are not pronounced at "pasteurization" levels. Beef has been shown to be more susceptible than pork to flavor and odor alteration (3, 5). In the present study, both antibiotics and irradiation were used, when needed, to allow maximum flexibility in holding conditions with the objective of establishing the optimum aging time and temperature for the rapid tenderization of beef.

EXPERIMENTAL METHODS

Preliminary work was conducted in which the experimental procedures and the level of irradiation were established. In combination with antibiotic treatment 45,000 to 50,000 rad of gamma radiation was found to be sufficient to control microbial growth under the conditions employed. Although irradiation at higher levels was somewhat more effective, dosages of 100,000 rad or more resulted in detectable flavor changes in the aged steaks.

Inside rounds from chilled U. S. Good and Utility beef carcasses, obtained approximately 72 hr post slaughter, were used in these studies. The rounds were stitch pumped to 106% of their weight with a solution containing an appropriate amount of antibiotic. The stitch pumping procedure was found to be effective when 30 to 50 ppm of antibiotic were infused into the meat prior to aging. It was assumed that the other methods which would provide completely uniform distribution would permit a lower level of antibiotic to be employed.

The *semimembranosus* muscle from the rounds was sliced into $\frac{3}{4}$ in steaks and allocated to the aging conditions in a randomized design so that all steak locations, left and right sides and carcasses were, as far as possible, represented in all treatments of a particular experiment. The beef was aged in the form of steaks which allowed adjacent locations within a muscle to be subjected to different treatments. This procedure was chosen because it most nearly approached using identical samples for two treatments and, in addition, took advantage of the similarity between sides. Previous work (1) in this laboratory had shown that steaks 4–9 (anterior to posterior) were the most uniform in tenderness and these were used in the aging experiments.

A surface dressing on each steak was obtained by placing the steak in a plastic bag (Cry-O-Vac)^e containing 50–100 ml of pumping solution. The bag was agitated, allowed to stand for 30 min and the fluid content decanted prior to sealing the package under vacuum. All preparation work was conducted at 45° F.

One experiment was designed to determine whether irradiation would interfere with tenderization at elevated aging temperatures. In this instance the packaged steaks were held overnight at 35° F and then transported to the Argonne National Laboratory in a refrigerated container. Irradiation was performed in a measured gamma field obtained from spent fuel rods. Refrigeration sufficient to maintain a steak temperature of 35° to 40° F during irradiation was provided by refrigeration plates. This preparatory work was completed in approximately 30 hr.

Aging at 90°, 100°, 110° and 120° F was conducted in convection type incubators, all steaks being at the same height in the incubator. For aging at 35° F, a forced air cooler was used. Unaged control steaks were frozen at –20° F immediately following cutting.

A bacteriological examination of the steaks was made after aging. A weighed amount of meat was removed aseptically from a steak and homogenized in a Waring blender in sterile distilled water. Aerobic counts were made by the conventional quantitative plating technique using tryptone-yeast extract-glucose

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^e Supplied by the CRYOVAC Company.

agar (2), incubated for 48 hr at 86° F (30° C). Populations of anaerobic bacteria were estimated by adding 0.1 ml of a suitable dilution of the meat infusion to 20 ml of medium in a screw cap tube. Brewer's medium (Difco), modified to contain 0.1% soluble starch was employed. The cultures were incubated for 3 days at 86° F (30° C). Although it was realized that this technique lacked precision because of growth by facultative bacteria, it served the purpose for which it was intended in these experiments.

A further test for the presence of toxic substances was made by inoculating mice intraperitoneally with 0.2 ml of the filtrate from the water and meat homogenate used for plate counts.

After the samples for bacteriological analysis were removed, the steaks were wrapped in aluminum foil and frozen. When the bacteriological examination was completed the steaks were thawed overnight at 45° F prior to broiling. Cooking and sampling procedures were the same as described in earlier reports (1, 8). Initial tenderness of the steaks and the amount of residue remaining after chewing were recorded using a scoring range from 1-10 corresponding to increasing tenderness and decreasing residue. Panel data were analyzed by analysis of variance or Student's "t" test. For shear measurements, 5-7 cores, 1/2 in in diameter, were cut from the portion of the cooked steaks not used for the taste panel. The cores were brought to 45° F and sheared perpendicular to the fibers using a Warner-Bratzler shear machine.

Specific procedures applying to individual experiments are given prior to the results and discussion of each.

RESULTS AND DISCUSSION

Effect of gamma irradiation on tenderness. In a preliminary experiment, irradiation in excess of 100,000 rad had an apparent adverse effect on the tenderness of the steaks during subsequent aging. The following experiment was designed therefore, to determine whether irradiation at 45.5 thousand rad had a significant direct effect on the tenderness of steaks or on tenderization at elevated temperatures. An aging temperature of 110° F was used. Matched inside rounds of 8 U. S. Good carcasses were infused with oxytetracycline,¹ 2 pairs with 20 ppm and 6 pairs with 30 ppm. After cutting into steaks and packaging, one half of the steaks were irradiated. One half of the steaks from the irradiated group and one half from the non-irradiated group were randomly allocated to an aging temperature of 110° F for 24 hr and the remainder were frozen immediately as controls.

Microbiological control of both irradiated and non-irradiated steaks was generally satisfactory. Aerobic counts averaged less than 100,000 organisms per g and the anaerobic counts were usually lower than the aerobic counts. The irradiated steaks had slightly lower aerobic and anaerobic counts but the antibiotic treatment alone yielded satisfactory control during aging and was used subsequently when aging was carried out at 110° F. The relative effectiveness of gamma irradiation was more pronounced in subsequent experiments when the temperature was more nearly optimal for bacterial growth.

The mean tenderness and residue scores, shear values, and their statistical analyses are given in Tables 1a and 1b respectively.

Taste panel results confirmed preliminary observations that irradiation of steaks at a level of 45,500 rad

TABLE 1a
Tenderness values of steaks receiving 45.5 thousand rad of gamma radiation prior to aging at 110° F

Treatment	Hours (at 110° F)	No. of judgments	Tenderness		Shear ¹ (lb)
			Initial	Residue	
Control.....	0	180	5.3	5.9	10.1
Control.....	24	183	6.6	6.7	9.2
Irradiated.....	0	185	5.4	6.0	10.1
Irradiated.....	24	185	6.4	6.6	9.4

¹ Mean of 24 steaks, 5-7 determinations per steak.

had no adverse effect on the flavor of the product. This level of irradiation did not affect the tenderness of unaged steaks or inhibit tenderization when steaks were subsequently aged at 110° F. Significant increases in tenderness were found in steaks aged at 110° F for 24 hr.

Aging at 90° F. Matched inside rounds from 8 U. S. Good carcasses were infused with oxytetracycline (OTC). The first 2 pairs received 30 ppm OTC but because microbial control was not entirely satisfac-

TABLE 1b
Analysis of variance for tenderness and residue scores and shear values of irradiated steaks aged at 110° F

Source of variation	Tenderness			
	df	Initial ms	Residue ms	Shear ms
Irradiation.....	1	0.03	0.79	0.97
Animal.....	7	0.88	1.02	16.51***
Aging.....	1	29.48***	16.42***	17.10***
Residual.....	86	0.80	1.43	2.25

*** P<0.001.
df—degrees of freedom.
ms—mean square.

tory, the remaining 6 pairs received 50 ppm OTC. After packaging, the steaks received approximately 45,500 rad of gamma radiation and were aged 0, 1, 2, or 3 days at either 35° or 90° F.

Microbial growth was satisfactorily controlled in this experiment. The anaerobic counts ranged from 1 x 10³ to 240 x 10³ organisms per g of meat depending on the aging time.

The tenderness and residue scores, shear values, and the analysis of the results are shown in Tables 2a and 2b, respectively. Steaks held at 90° F for 1, 2, or 3 days had significantly higher tenderness and residue scores than unaged controls. Some increases in tenderness were noted in the steaks held at 35° F but in

TABLE 2a
Effect of time and temperature of aging on the tenderness scores and shear values of semimembranosus steaks

Days aged	Tenderness ¹				Shear values ²	
	Initial		Residue		35° F	90° F
	35° F	90° F	35° F	90° F	(lb)	(lb)
0	5.5	5.4	5.6	5.5	10.1	9.9
1	6.0	6.0	6.1	6.2	9.9	9.7
2	5.7	6.5	5.6	6.5	10.4	8.7
3	5.9	6.2	5.8	6.2	9.7	9.3

¹ Mean of 96 judgments for each time-temperature group.

² Mean of 12 steaks, 5-7 values per steak.

¹ Supplied by Chas. Pfizer and Co., Inc.

TABLE 2b
Analysis of tenderness scores and shear values of steaks aged at 90° F

Source of variation	df	Tenderness		Shear	
		Initial ms	Residue ms	df	ms
Time.....	3	15.17**	13.11**	3	8.60
Temp.....	1	9.99	15.05*	1	66.17*
T x T.....	3	11.04**	9.50*	3	19.21
Residual.....	756	2.84	2.83	625	10.27
L.S.D. .05.....		.50	.49		1.00
L.S.D. .01.....		.64	.64		1.30

* P<0.05.

** P<0.01.

general these differences were not significant. Differences in shear values were significant only for temperature effect. Because temperature had a significant effect on residue scores and not on initial tenderness scores may indicate that the Warner-Bratzler shear is a better measure of changes in connective tissue than of changes in muscle fibers during aging. The greatest increase in tenderness, as determined by the three measurements of tenderness, was in those steaks which were held for 2 days at 90° F. It will be observed in Table 2a that the steaks reached an apparent optimum tenderness after 2 days. A similar effect occurred at other aging temperatures and is discussed in conjunction with the experiment which follows.

Aging at 110° F. In this experiment, 4 pairs each of U. S. Good and Utility inside rounds were infused with 50 ppm of oxytetracycline and prepared for aging as previously outlined. U. S. Good rounds were used in the second half of this experiment because it was thought that any aging effects in the steaks from Utility grade rounds were being partially masked by the large amount of connective tissue noted by the panel members. The steaks were aged 0, 16, 24 or 40 hr at 35° to 110° F.

With the exception of the steaks which were aged 40 hr at 110° F the bacteria counts on samples of the aged product were generally low. Six of the 12 steaks aged for 40 hours at 110° F had apparent anaerobic counts in excess of 100,000 organisms per g and were positive for staphylococci. Taste panel judgments were not made on these steaks.

The taste panel and shear test results on the steaks from U. S. Good and Utility rounds are shown in Table 3a. The analysis of these data appears in

Table 3b. The tenderness of the steaks aged at 110° F increased significantly at all aging times. The steaks which showed the greatest difference in tenderness compared to the controls held at 35° F were those which were aged 24 hr at 110° F. These differences were apparent in tenderness and residue scores as well as shear test values. A marked difference in taste panel scores and shear values was obtained between grades but the extent of tenderization within each of the grades was quite comparable. As indicated previously, aging of steaks in this manner produced an apparent optimum tenderization followed by some loss of tenderness.

In this experiment evaporation losses during storage, defrosting, and cooking were noted. Moisture losses from the steaks aged 40 hr at 35° and 110° F averaged 6.2 and 17.6%, respectively. These losses were approximately 22 and 7% greater than occurred

TABLE 3b
Analysis of variance of taste panel scores and shear values of steaks aged at 35° F and 110° F

Source of variation	df	Tenderness		Shear	
		Initial ms	Residue ms	df	ms
Temperature.....	1	286.3***	36.6***	1	93.2***
Time.....	3	84.0***	5.3*	3	73.1***
Grade.....	1	113.0***	34.6***	1	5164.2***
Residual.....	636	2.4	2.47	512	10.24

* P>0.05.

*** P>0.001.

after 24 hr aging. Cooking losses were less in those steaks which were aged at 110° F but the total losses were 8% higher in steaks aged for 40 hr than they were for steaks aged for 24 hr. It is felt that the greater moisture loss in the steaks aged for 40 hr at 110° F was at least in part responsible for the lower tenderness scores of these steaks. Weight losses in these experiments could be expected to be higher than under conditions of normal aging because of the moisture added in the antibiotic treatment.

Bacterial spoilage was more conveniently controlled at 110° F than at 90° F. In addition, greater tenderization was noted when steaks were aged at the higher temperature and shorter time. For these reasons 110° F was accepted as being more nearly the optimum for aging and a further experiment was de-

TABLE 3a
Mean tenderness scores and shear values of steaks from Utility and Good rounds aged at 110° F

Grade	Aging time	No. of judgments	Tenderness				Shear values	
			Initial		Residue		35° F	110° F
			35° F	110° F	35° F	110° F		
Utility	(hr)						(lb)	(lb)
	0	42	4.6	5.7	5.2	6.2	14.7	14.5
	16	42	4.9	6.2	5.7	6.6	16.2	13.2
	24	47	5.3	7.4	5.6	7.1	13.7	12.5
	40	22	4.7	5.7	4.8	6.0	15.6	13.2
Good	0	48	5.9	5.9	6.4	6.5	7.6	7.7
	16	48	6.3	6.9	6.4	7.0	8.7	7.8
	24	48	6.5	7.5	6.8	7.2	8.1	7.1
	40	24	5.9	6.7	6.5	6.9	7.7	7.7

signed to investigate aging temperatures within a 10° F range of the apparent optimum.

Aging at 100° F, 110° F and 120° F. Matched pairs of the inside rounds of six U. S. Good carcasses containing 50 ppm oxytetracycline were sliced and the steaks packaged, aged and stored according to the procedures previously described. Steaks aged at 100° F were held 2 days and those aged at 110° F and 120° F were held for 24 hr. Control samples were frozen immediately after packaging or after irradiation. The steaks that were aged at 100° F and their adjacent controls received 45.5 thousand rad of gamma radiation prior to aging. Bacterial growth was effectively controlled and all the steaks were submitted to the taste panel. Taste panel results and shear test scores are shown in Table 4. The differences between mean values of the various experimental treatments were analyzed by the 't' test and significant differences are indicated in the table.

As indicated in Table 4 initial tenderness and residue scores were related directly to the aging temperatures and were increased significantly over comparable unaged controls at all temperatures. In the lower portion of Table 4 comparisons are drawn between three aging conditions. The difference between mean scores for steaks aged at 100° F and 110° F are not significant and are in both instances significantly lower than those of steaks aged at 120° F. Although

TABLE 4
Mean tenderness, residue and shear values for steaks aged at 100°, 110°, and 120° F

Time and temp. of aging	No. of judgments	Tenderness		Shear	
		Initial	Residue	No.	(lb)
2 days at 100° F	114	6.0	6.1	77	8.75
Unaged controls	114	5.3**	5.5**	69	9.58NS
1 day at 110° F	112	6.3	6.2	84	8.69
Unaged control	112	5.1	5.2	89	9.73
1 day at 120° F	106	7.4	7.2	73	7.04
Unaged control	106	5.2***	5.4***	83	9.50***
2 days at 100° F	114	6.0	6.1	77	8.75
1 day at 110° F	112	6.3	6.2	84	8.69NS
2 days at 100° F	114	6.0	6.1	77	8.75
1 day at 120° F	106	7.4***	7.2	73	7.04***
1 day at 110° F	112	6.3	6.2	84	8.69
1 day at 120° F	106	7.4***	7.2***	73	7.04***

* P>0.05.
** P>0.01.
*** P>0.001.

the scores alone showed that 120° F was the preferred aging temperature, steaks aged in this manner were reported by the panel to be dry and to have a "warmed-over" flavor. These comments by the panel members and the partial destruction of the normal red color during aging suggested that the steaks were partially cooked and that only a part of the tenderization resulted from an aging effect. Aging at 100° F was eliminated because bacterial spoilage was more difficult to control than at 110° F. The above series of experiments demonstrated that steaks could be tenderized in the manner prescribed and it was of interest to determine if the tenderization achieved was

comparable to that obtained when steaks were aged for two weeks at 35° F.

High temperature aging compared to a more conventional aging procedure. Steaks were cut from 8 pairs of U. S. Good inside rounds which had been infused with oxytetracycline, 2 pairs with 20 ppm, and 6 pairs with 30 ppm. The increases in tenderness of steaks aged 24 hr at 110° F were compared to adjacent steaks aged 14 days at 35° F. The steaks were randomly assigned taking into consideration the differences between animals, sides, locations within the muscle and aging temperature.

After aging at 110° F, steaks from two of the rounds infused with 20 ppm of oxytetracycline had somewhat higher bacterial counts than those infused with 30 ppm but all were considered satisfactory for taste panel evaluation. The taste panel and shear test results are given in Table 5. Significant differences between means are indicated.

TABLE 5
Mean tenderness, residue and shear values for steaks aged at 35° and 110° F

Time and temp. of aging	No. of judgments	Tenderness		No. of cores	Shear
		Initial	Residue		
Unaged control—(35° F)	165	5.2	5.2	81	11.2
Unaged control—(110° F)	178	5.3	5.5	80	10.8
14 days—(35° F)	166	5.9	5.8	81	10.8
Unaged control	165	5.2**	5.2**	81	11.2
1 day—(110° F)	179	6.3	6.2	78	9.3
Unaged control	178	5.3***	5.5***	80	10.8**
14 days—(35° F)	166	5.9	5.8	81	10.8
1 day—(110° F)	179	6.3*	6.2*	78	9.3**

* P<0.05.
** P<0.01.
*** P<0.001.

Tenderness was increased significantly by both aging treatments. The mean tenderness scores showed an increase of 0.7 unit on the tenderness scale for aging at 35° F and 1.0 unit for aging at 110° F. Shear values were unchanged by aging at 35° F but followed the taste panel scores for steaks aged at 110° F. The results of the Warner-Bratzler shear test in this experiment were rather typical of other comparisons in which the larger taste panel differences in tenderness were reflected in significant differences in shear values and small differences were not. From the results given in Table 5 it was concluded that under the conditions used, steaks were as effectively tenderized in 24 hours at 110° F as in 14 days at 35° F.

While this study was primarily concerned with tenderness, an objectionable flavor, described by the panel members as acrid or stale, was frequently noted in the aged steaks. The undesirable flavor occurred more frequently in steaks aged at the higher temperature. It seems likely that the large surface area of the steaks provided a greater opportunity for flavor deterioration than would the relatively limited surface area of beef rounds or sides.

SUMMARY

Round steaks from U. S. Utility and U. S. Good carcasses were used to determine the optimum conditions of high temperature and time for increasing the tenderness of beef by aging. Microbial growth during aging was effectively controlled by infusion of 30-50 ppm of oxytetracycline into the rounds prior to cutting, or by 45,500 rad of gamma radiation in combination with the antibiotic treatment.

At the level used (45,500 rad), gamma irradiation did not affect the tenderness or tenderization of steaks aged at 110° F for 24 hours.

In other experiments, 2 days at 90° F and 24 hours at 110° F were found to be optimal for aging steaks to achieve effective tenderness. Steaks aged for 24 hours at 120° F showed greater increases in tenderness than those aged for 24 hours at 110° F or for 2 days at 100° F. Aging at 90° F necessitated more severe treatment to prevent excessive bacterial growth; while aging at 120° F resulted in more undesirable color and flavor changes in the steaks than found in those aged at the lower temperatures. On this basis, 24 hours at 110° F was selected as the most desirable aging time and temperature for the rapid tenderization of beef round steaks. Additional studies showed that steaks aged for 24 hours at 110° F had tenderness increases comparable to those found in steaks aged for 14 days at 35° F.

LITERATURE CITED

1. GINGER, BETTY, AND WEIR, C. EDITH. Variations in tenderness within three muscles from beef round. *Food Research*, 23, 662 (1958).
2. HAYNES, W. C., WICKERHAM, L. J., AND HESSELTINE, C. W. Maintenance of cultures of industrially important microorganisms. *Appl. Microbiol.*, 3, 361 (1955).
3. KIRN, J. F., URBAIN, W. M., AND CZARNECKI, H. J. Characteristics of electron-irradiated meats stored at refrigerator temperatures. *Food Technol.*, 10, 601 (1956).
4. NIVEN, C. F. JR., AND CHESBRO, W. R. Complementary action of antibiotics and irradiation in the preservation of fresh meats. *Antibiotics Annual 1956-1957*, New York Medical Encyclopedia, Inc., p. 855.
5. SCHULTZ, H. W., CAIN, R. F., NORDAN, H. C., AND MORGAN, BRUCE H. Concomitant use of radiation with other processing methods for meat. *Food Technol.*, 10, 233 (1956).
6. SLEETH, R. B., HENDRICKSON, R. L., AND BRADY, D. E. Effect of controlling environmental conditions during aging on the quality of beef. *Food Technol.*, 11, 205 (1957).
7. SLEETH, R. B., KELLEY, G. G., AND BRADY, D. E. Shrinkage and organoleptic characteristics of beef aged in controlled environments. *Food Technol.*, 12, 86 (1958).
8. WEIR, C. EDITH, WANG, H., BIRKNER, MARION L., PARSONS, J., AND GINGER, BETTY. Studies on enzymatic tenderization of meat. II. Panel and histological analyses of meat treated with liquid tenderizers containing papain. *Food Research*, 23, 411 (1958).
9. WEISER, H. H., GOLDBERG, H. S., CAHILL, V. R., KUNKLE, L. E., AND DEATHERAGE, F. E. Observations on fresh meat processed by the infusion of antibiotics. *Food Technol.*, 7, 495 (1953).